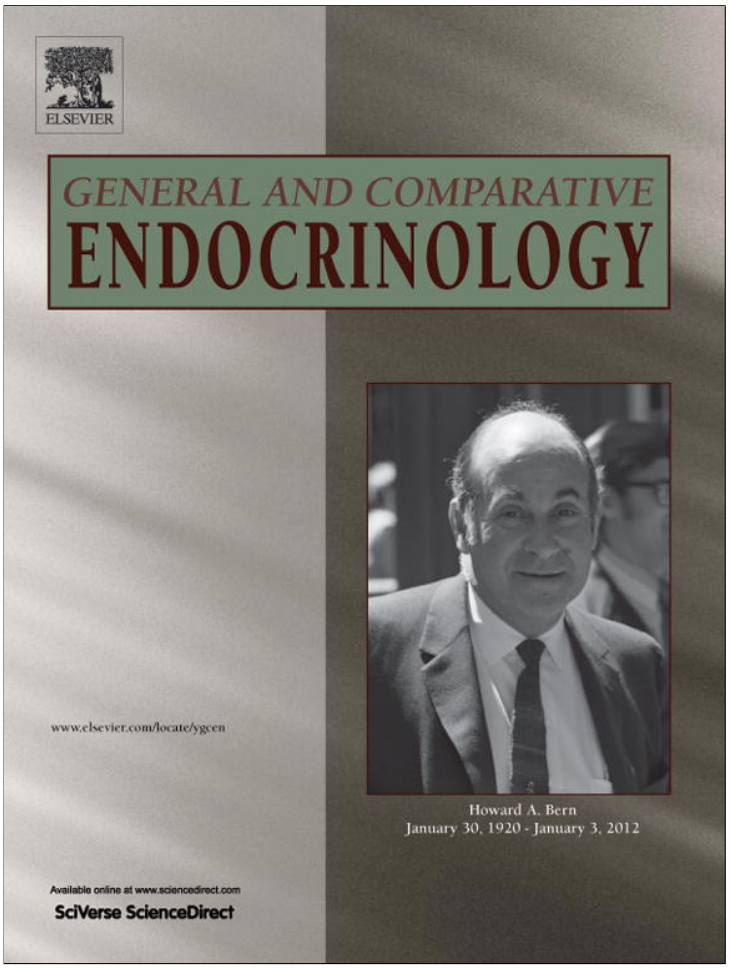

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Experimental increase of testosterone levels in free-ranging juvenile male African striped mice (*Rhabdomys pumilio*) induces physiological, morphological, and behavioral changes

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ABSTRACT

Testosterone influences sexual differentiation in early development, and activates sexual maturation and sex-related behavior in males during puberty. Testosterone can also influence the expression of male alternative reproductive tactics, by either organizational effects (fixed tactics) or by activational effects (plastic tactics). However, the roles of testosterone in sexual maturation and at the same time the expression of alternative reproductive tactics have been little investigated experimentally, and studies of free-ranging mammals are lacking. We conducted a field experiment in free-ranging juvenile African striped mice (*Rhabdomys pumilio*), a species with alternative reproductive tactics. Juvenile male striped mice reaching puberty can remain in their family as philopatric group-living males with low testosterone levels, or they can disperse and become solitary living roamers with much higher testosterone levels. We tested whether experimentally increased testosterone levels in non-scrotal juvenile males induces puberty and leads to the expression of the roaming tactic. Testosterone-treated males received the hormone for 15 days by silastic implants which were empty in control-treated males. When compared to control-treated males, testosterone-treated males had higher testosterone levels, lower corticosterone levels, and became scrotal with descended testes. Testosterone-treated males also had larger testes, larger epididymides, and showed indication of spermatogenesis. Testosterone-treated males did not become solitary-living roamers, but had larger home ranges than control males. We conclude that testosterone can induce sexual maturation and causes juvenile males to increase their home ranges, maybe to search for dispersal opportunities.

1. Introduction

Gonadal hormones like testosterone have two types of influences on physiology and behavior, termed "organizational" and "activational" effects [27]. Organizational effects of testosterone typically occur at early developmental stages (i.e. intrauterine life and neonatal period) leading to genital and brain sexual differentiation [2]. By contrast, activational effects occur later in life [2]. For instance, testosterone surges at puberty, leading to sexual maturation [22] and the expression of sex-related behavior [4]. Testosterone is important for sexual differentiation, but also for individual differences in morphology, physiology, and behavior within the male sex. The extent of these differences can be determined by both genetic [45,47] and environmental factors [51].

In many animal species, males exhibit alternative reproductive tactics (ARTs) [11], that is, discontinuous behavioral and other traits selected to maximize fitness in two or more alternative ways in the context of reproductive competition [23]. The relative plasticity hypothesis suggests that the development of ARTs is regulated by either organizational or activational effects of steroid hormones like testosterone [20,21]. In male species with permanent reproductive tactics, so called fixed ARTs [20], a given tactic can be influenced by early exposure to testosterone. In tree lizards, *Urosaurus ornatus*, gonadectomy and testosterone replacement performed at post-hatching determined future reproductive tactics [15], while such treatments performed later in life did not affect their tactics [14]. These authors concluded that testosterone influences reproductive tactic differentiation by its organisational effect. By contrast, males of other species can change their reproductive tactics later in life, indicating plastic ARTs [20]. By experimentally changing androgens

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levels in different species, previous studies demonstrated that these males changed reproductive tactics, i.e. they changed their reproductive behaviors such as territoriality, frequency of copulation, and courtship [9,19,24,25,26,44,46,50]. For example, territorial male marine iguanas, *Amblyrhynchus cristatus* treated with flutamide (a testosterone and dihydrostestosterone receptor blocker) to block the effects of testosterone, made them more similar to satellite males [50]. These males decreased their ability to defend a territory, which ultimately decreased their fitness [50]. This indicates that the development of reproductive tactics was induced by activational effects of androgens. Experimental studies testing the effects of androgens on the expression of ARTs were mainly conducted in fish and reptile species, and we report here the first field experiment on the role of testosterone in a mammalian species showing male ARTs.

The African striped mouse, *Rhabdomys pumilio*, is a socially flexible species [34], that is, both males and females can change their reproductive tactics as a response to changing environmental conditions leading to changes in the social system. Three male ARTs have been identified that differ in steroid hormone levels [38] and in reproductive success [34]: (i) philopatric group-living males with low testosterone and high corticosterone levels and very low reproductive success, (ii) solitary-living roamers with high testosterone and low corticosterone levels and intermediate reproductive success, (iii) dominant group-living territorial breeders with intermediate testosterone and low corticosterone levels and high reproductive success. Males that change their tactic also change their hormone profile. For example, philopatric group-living males that become solitary-living roamers have increased testosterone levels after the change in tactic [36]. Philopatric group-living males have to disperse from their natal group to become either solitary-living roamers or dominant group-living territorial breeders; they cannot become the breeding male in their natal group [35,38]. Males can disperse as juveniles at 4 weeks of age to become solitary-living roamers [32], or alternatively they become philopatric group-living males [34]. Importantly, juvenile males do not directly become dominant group-living territorial breeders, because the latter are larger and more dominant than juvenile males [35,38]. In sum, when juvenile males can undergo sexual maturation (four weeks old), they can either become philopatric group-living males with low testosterone levels or solitary-living roamers with high testosterone levels, indicating that the development into solitary-living roamers might be regulated by an increase in testosterone levels [36].

We examined the effects of increased testosterone levels on phenotypic traits in juvenile male striped mice. We focused on traits that differ between philopatric group-living males and solitary-living roamers. Compared to solitary-living roamers, philopatric group-living males are more often non-scrotal (proportion of non-scrotal philopatric group-living males: 28.84% vs. proportion of non-scrotal solitary-living roamers: 0% [38], have smaller testes when being scrotal [33], and show higher corticosterone levels [38] due to environmentally- and socially-induced sexual suppression [39]. High glucocorticoid levels appear to inhibit steroidogenesis of androgens, spermatogenesis and gonad development [29]. High testosterone levels can alter the hypothalamus pituitary adrenal (HPA) axis response to stress as demonstrated in male Sprague Dawley rats, *Rattus norvegicus*, [17,18,49] and in men [30]. Gonadal steroids such as testosterone can also modulate basal corticosterone levels [16]. For example, in Sprague–Dawley male rats, gonadectomised males showed higher basal corticosterone levels than control males, while gonadectomised males with testosterone or dihydrotestosterone replacement showed a return to the basal corticosterone levels of intact males [43]. This suggests that the interaction of the hypothalamus pituitary gonadal (HPG) axis with HPA might mediate gonadal functions [12,48]. In striped mice, increased testosterone levels could thus lead to decreased corticosterone secretion and the activation of the HPG axis, starting gonadal function. We thus predicted that juvenile males with testosterone implants would undergo sexual maturation indicated by lower basal corticosterone levels, larger testes, larger epididymides, and higher spermatogenesis activity than control males.

A difference between philopatric and roaming African striped mouse males is that philopatrics are group-living while roamers are solitary-living. Furthermore, solitary-living roamers have larger home ranges than dominant group-living territorial breeders, which in turn have larger home ranges than philopatric group-living males [37]. Experimental increases of circulating androgen levels can lead to increased home range sizes such as in side-blotched lizard males (*Uta stansburiana*) [10], Galapagos marine iguana males (*A. cristatus*) [50], and bank voles (*Myodes glareolus*) [19]. We thus predicted that juvenile males with testosterone implants would first expand their home ranges, searching for dispersal and mating opportunities, and then leave their natal group and start solitary living.

2. Materials and methods

2.1. Study area

Striped mice were studied during the breeding season 2010, from September to November. Our field site of 17 ha was located on the farm Klein Goegap (29°42.30′S–18°02.95′E) in the Succulent Karoo of South Africa.

2.2. Trapping and marking of animals

Reproductive status and body mass were monitored by trapping striped mice directly at their nests by using metal live traps similar to Sherman's traps ($26 \times 9 \times 9$ cm) baited with a mixture of bran flakes and salad oil. Males were recorded as being either scrotal (testes fully descended) or non-scrotal. Mice were permanently marked using numbered metal ear tags (National Band and Tag Co., Newport, KY, USA). Additionally, each individual was dyed for visual identification with a mark on the pelage (Rapido, Pinetown South Africa). Group composition was determined both by direct nest observations at least 1 day a week and by radiotracking at night (see below). Nests were observed twice a day during the morning and evening for 30 min each, and the presence of all marked individuals was recorded [40].

2.3. Experimental hormone manipulation

Non-scrotal juvenile males were captured for study when they achieved a body mass of 19–30 g, at approximately four weeks old [38], the earliest age for dispersal [32]. Study subjects were anaesthetized with di-ethyl ether and the subcutaneous implantation was performed behind the neck with a precision 10 gauge trochar (Innovative Research of America, Sarasota, FL, USA). They received a 4 mm silastic implant (inner diameter 0.147 cm, outer diameter 0.196 cm) randomly containing either 0.2 mg of testosterone (Acros Organics, New Jersey, USA) or being empty. Ten juvenile males received the testosterone implant (testosterone-treated males) and eight others received an empty implant (control-treated males). Eight testosterone-treated males and six control-treated males originated from six groups with a maximum of two testosterone-treated males and one control-treated male per litter in a group. Two testosterone-treated males and two control-treated males originated from three solitary-living females with a maximum of one testosterone-treated male and one control-treated male per litter.

2.4. Determination of home ranges and sleeping sites

All testosterone-treated and control-treated males were equipped with MD-2C radiotransmitters weighing 1.2 g (Holohil, Carp, Ontario, Canada). Radiotracking was performed with an AOR 8000 wide-range receiver (Tokyo, Japan) and an H-antenna (Africa Wildlife Tracking, Pretoria, South Africa). Home ranges were determined for eight non-consecutive days by radiotracking striped mice six times per day from 9:00 to 12:00 am and from 2:00 to 5:00 pm when striped mice are most active [41]. The accuracy of the GPS device (eTrex Venture, GARMIN International, Olathe, KS, USA) was ±6 m at our field site. To calculate home range sizes, we used the convex polygon (100% cores) method with the RANGES6 software.

Composition of sleeping groups was determined by radiotracking at night when mice are inactive. For this, 10 breeding females (including the eight mothers of studied subjects) and 10 dominant group-living territorial breeders were also equipped with MD-2C radiotransmitters weighing 2 g (Holohil, Carp, Ontario, Canada). Individuals were considered as solitary-living when they slept alone for at least 70% of the time and group-living when they slept more than 50% of the time with at least one other adult individual [37].

The duration of increased testosterone levels was set to 14 days because this time period was considered to be long enough to allow juvenile males to explore their environment and disperse. Juvenile philopatric striped mouse males typically increase their home range for about 1 week before becoming solitary-living roamers (Schradin, unpublished data).

2.5. Blood samples and tissue collection

Blood samples were collected in the morning when individuals emerged from their nest between 7:00 and 8:00 am, controlling for possible circadian rhythms of hormone release. As soon as a striped mouse entered a trap, it was removed and anaesthetized with diethyl ether and a blood sample of about 200 μl was collected from the sub-lingual vein [13] within less than 3 min. After 1 h, blood samples were centrifuged for 10 min (1000 rpm; Biofuge pico, Kendro Laboratory Products), the serum pipetted and centrifuged again to remove any blood cells, then frozen in aliquots of 50 μl for testosterone and of 10 μl for corticosterone assays.

For each testosterone-treated and control-treated male, we collected a first blood sample on D-1, i.e. the day before the implantation (D0). We collected a second and a third blood sample 6 days (D6) and 14 days (D14) after the implantation. Due to predation (for details see Section 2.8), we only collected a second blood sample for seven of the ten testosterone-treated males and six of the eight control-treated males. A third blood sample was obtained for five testosterone-treated males and six control-treated males. After the third blood sample, males were sacrificed by asphyxia to collect their testes and epididymides (five treated and six control), which were weighed and frozen at −20 °C. For one of the testosterone-treated males, testes and epididymides were collected immediately after the second blood sample at the end of the field experiment.

2.6. Hormone assays

For testosterone and corticosterone, we used commercial kits (IBL Hamburg, Germany) that had previously been validated for striped mouse serum [31]. Since corticosterone levels are very high in philopatric group-living males [38], samples for the corticosterone assay were diluted 1:24. Eight out of 38 samples had a too small volume for testosterone measurements and had to be diluted with a zero standard. All 38 samples were analyzed within a single assay for testosterone and a single assay for corticosterone. All samples were run in duplicate. The intra-assay coefficients of variation, calculated from the coefficients of variation from the samples measured in duplicate, were 2.95% for testosterone and 7.66% for corticosterone.

2.7. Sperm counts and cell cycle stages in testes

Testis and epididymis samples were analyzed at the Leibniz Institute for Zoo and Wildlife Research (Berlin, Germany). For the determination of the number of testicular spermatozoa, testis parenchyma from the outer third of the testis (avoiding the rete testis region) was cut with a sharp razor blade into small pieces. Testis parenchyma was weighed, minced and suspended in 2 ml Dulbecco's buffered saline (Sigma D 8537) while carefully pressing through a 28 μm nylon mesh. After appropriate dilution in water, the sperm concentration was counted in a haemocytometer and given as the total sperm number.

For the analysis of cell cycle stages in the testes, frozen testis parenchyma was thawed, testicular cells were dispersed and flow cytometric DNA analysis was applied according to Blottner et al. [5]: testis parenchyma was fine minced in 1 ml 100 mM citric acid containing 0.5% (v/v) Tween 20 and agitated for 20 min at room temperature. The DNA was stained by adding 4 ml of a 400 mM Na_2HPO_4 solution containing 5 μM 4′,6-diamidino-2-phenylindol (DAPI) for 10 min in the dark. Measurements were performed on a PAS III flowcytometer (Partec, Germany) equipped with a mercury lamp (excitation: 360 nm, emission: 420 nm). Cells were counted and the histograms were analyzed for the proportions of cells in each peak by the FlowMax software (Partec, Germany). Haploid signals (1C) come from postmeiotic germ cell stages like spermatids and sperm cells, diploid signals (2C) from spermatogonia, secondary spermatocytes and somatic testicular cells, (4C) tetraploid signals mainly derive from G2/M phase of cell cycle in meiotic primary spermatocytes but also in mitotic spermatogonia. Between 2C and 4C, cells in the S-phase (S) were detected. Germ cell transformations were evaluated with the calculation of the diploid/tetraploid ratio (2C/4C), haploid/diploid ratio (1C/2C), and haploid/tetraploid ratio (1C/4C).

2.8. Data analysis

Due to natural predation, samples sizes were reduced. Predation was ascertained with certainty. We found two radiotransmitters in snake feces (snake predators were daily radiotracked until radiotransmitters were recovered) and we found one fresh dead male with a snake bite to the body. We found one radiotransmitter on the ground close to a male's nest (the wire mesh of the transmitter was found intact, indicating that this male did not loose its transmitter but was eaten). One male got a signal from its transmitter coming from the crack of a small hill, indicating that this male was taken by a bird of prey. Three testosterone- and two control-treated males were predated before the second blood sample. One testosterone-treated male was predated before the third blood sample. Thus, data from testosterone-treated males and control-treated males were considered for statistical analyses when we could collect at least a second blood sample for these males.

Home range data were available for six testosterone-treated males and six control-treated males. The maximum of available days of radiotracking were (t: test males/c: control males): 8 days (t: 2/c: 4), 7 days (t: 1), 5 days (t: 1/c: 2), 4 days (t: 2). For males with less than 8 days of radio-tracking data, we calculated expected home ranges for day 8. For this, we multiplied home-range size on the last day of radiotracking by the percentage by which home ranges increased from the last day of radiotracking to 5, 6, 7, and 8 days of radiotracking in the remaining males of the study (for the same approach see [41]. Thereafter, we calculated slopes and intercepts of the relationships between home ranges and days

of radiotracking for each testosterone-treated and control-treated male before and after the home range corrections using linear regression functions. We therefore showed that there was no significant difference in slope values before ($mean_{slope}$ = 0.06 ± 0.05) and after home range corrections ($mean_{slope}$ = 0.05 ± 0.03) (exact Wilcoxon signed rank test: N = 12; V = 15; p = 0.70). Additionally and for comparison, we obtained home range data for seven of the ten breeding males equipped with radio-collars.

Statistical analyses were carried out with R 2.12.2 [28]. Results are presented as mean ± SD and significance was accepted at $\alpha \leqslant 0.05$. We used non-parametric statistical analyses due to small sample sizes. Pairwise or multiple comparisons between D-1, D6, and D14 within an experimental group (i.e. testosterone-treated or control-treated males) were performed with paired exact Wilcoxon signed rank tests and Friedman rank sum tests. Comparisons between home ranges and body mass of dominant group-living territorial breeders with testosterone-treated males and with control-treated males were performed with unpaired Exact Wilcoxon Rank Sum Tests (equivalent to Mann–Whitney U test with R2.12.2). Comparisons between testosterone-treated and control-treated males were also performed with unpaired Exact Wilcoxon Rank Sum Tests and Fisher's Exact Tests. To test for a relationship of testosterone levels with corticosterone levels, we performed a Spearman correlation. For these correlations, data for testosterone-treated and control-treated males were combined.

To test for the effect of the treatment on the testis and epididymis weights we used two Generalized Linear Models (GLM) fitted with a quasi binomial error distribution (N = 12; six testosterone-treated and six control-treated males). Testis mass was modeled following a proportion data analysis [8] by binding together the vectors of testis mass and body mass (i.e. male body mass corresponding to the day of testis collection) into a single object considered as a response. The response variable was also squared root transformed to achieve linearity of residuals. Epididymis mass was analyzed following the same procedure for testis mass. Finally, the effect of the treatment on whether testosterone-treated and control-treated males slept alone was analyzed with Generalized Linear Models (GLM) fitted with a quasi binomial error distribution (N = 12; six test and six control males). The number of times test and control males were sleeping with another member in their group were modeled with a proportion data analysis by binding together the vector of the number of times testosterone-treated and control-treated males slept alone and the vector of the total number of times testosterone-treated and control-treated males were radiotracked for sleeping site determination into a single object considered as a response.

3. Results

3.1. Description of the study population

Nine groups (with 14 females born the previous breeding season) and five solitary breeding females were present on our field site during the experiment. The ratio of solitary- to group-living females was 0.36. One of these 19 adult females produced two litters, and seven adult females produced a single litter, while eleven adult females (58%) did not reproduce at all. All ten adult males were scrotal and nine of these males were dominant group-living territorial breeders while one male followed a roaming tactic. We only observed one adult philopatric male that was group-living at the beginning of the breeding season.

3.2. Testosterone levels in juvenile males

Testosterone-treated and control-treated males showed similar testosterone levels before the treatment on D-1 (0.42 ± 0.59 ng/ml vs. 0.18 + 0.27 ng/ml; W = 27; p = 0.42; Fig. 1). Testosterone-treated males had significantly higher testosterone levels than control-treated males on D6 (8.32 + 4.45 ng/ml vs. 0.28 + 0.33 ng/ml; W = 42; p = 0.003) and on D14 (1.58 + 1.14 ng/ml vs. 0.14 + 0.22 ng/ml; W = 30; p = 0.006). We did not observe significant changes of testosterone levels among control-treated males during the experiment (Friedman chi-squared = 6.07; p = 0.30). Testosterone levels of testosterone-treated males increased significantly from D-1 to D6 (V = 0; p = 0.02), then decreased from D6 to D14 though not quite significantly (V = 15; p = 0.06; Fig. 1). The testosterone levels of testosterone-treated males did not increase significantly from D-1 to D14 (V = 3, p = 0.31).

3.3. Corticosterone levels in juvenile males

Testosterone-treated and control-treated males showed similar corticosterone levels before the treatment on D-1 (758.16 ± 394.41 ng/ml vs. 832.19 ± 524.73 ng/ml; W = 19; p = 0.84; Fig. 2). Testosterone-treated males had significantly lower corticosterone levels than control-treated males on D6 (649.45 ± 212.80 ng/ml vs. 1082.38 ± 268.28 ng/ml; W = 4; p = 0.01) but not on D14 (409.85 ± 157.15 ng/ml vs. 683.14.85 ± 463.29 ng/ml; W = 11; p = 0.54). We did not observe significant changes of corticosterone levels among control-treated males during the experiment (Friedman chi-squared = 6.24; p = 0.28). Corticosterone levels of testosterone-treated males did not decrease significantly from D-1 to D6 (V = 17; p = 0.69) nor from D6 to D14 (V = 10; p = 0.63). The corticosterone levels of testosterone-treated males decreased, though not quite significantly, from D-1 to D14 (V = 15; p = 0.06).

3.4. Correlations between testosterone and corticosterone levels in juvenile males

Testosterone and corticosterone levels were significantly negatively correlated at D6 (r_s = −0.58; p = 0.038) but not at D-1 (r_s = −0.16; p = 0.59) and D14 (r_s = −0.31; p = 0.35).

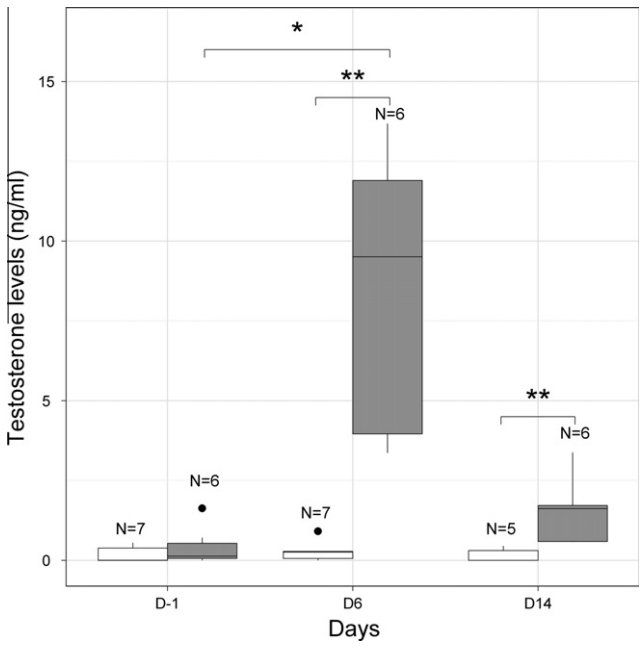

Fig. 1. Serum testosterone levels before (D-1), 6 days (D6), and 14 days (D14) after the testosterone treatment in testosterone-treated (grey) and control-treated males (white). The median is indicated by the horizontal bar inside the box, the first and third quartile by the box itself, and the horizontal bars outside the box indicate the minimal values and the fourth quartile (outliers are shown by a black-filled circle). Only significant pairwise comparisons are reported on the figure: $^{*}p < 0.05$; $^{**}p < 0.01$.

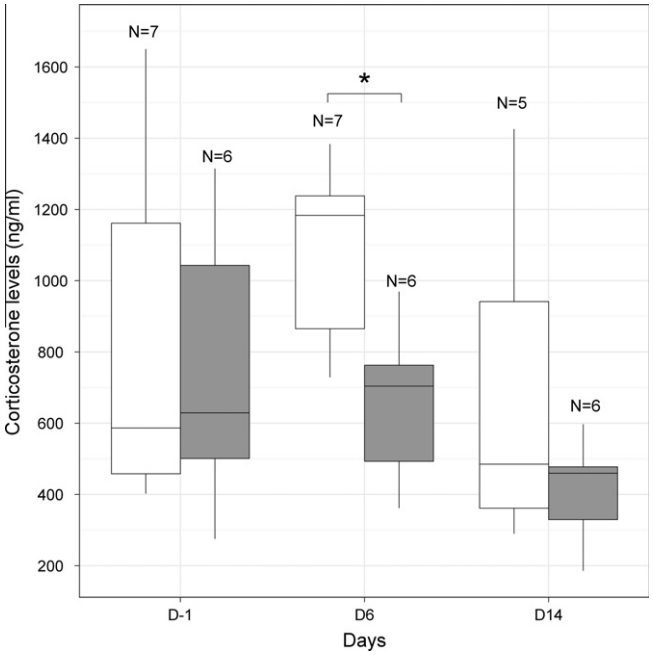

Fig. 2. Serum corticosterone levels before (D-1), 6 days (D6), and 14 days (D14) after the testosterone treatment in testosterone-treated (grey) and control-treated males (white). The median is indicated by the horizontal bar inside the box, the first and third quartile by the box itself, and the horizontal bars outside the box indicate the minimal values and the fourth quartile. Only significant pairwise comparisons are reported on the figure: $^{*}p < 0.05$.

3.5. Body mass

Testosterone-treated and control-treated males did not differ significantly in body mass, neither before ($W = 19$; $p = 0.83$) nor after the treatment (D6: $W = 24$; $p = 0.72$; D14: $W = 20.5$; $p = 0.36$; Table 1). The mean body mass of dominant group-living territorial breeders was two times greater than the body mass of testosterone-treated males (53.97 ± 3.39 g vs. 25.71 ± 3.25 g; $W = 49$; $p < 0.001$) and of control-treated males (53.97 ± 3.39 g vs. 25.00 ± 4.56 g; $W = 42$; $p < 0.01$) at the end of their testosterone or control treatment.

3.6. Reproductive parameters of juvenile males

All males were non-scrotal on D-1. On D6, two out of seven testosterone-treated males and none of the six control-treated males were scrotal (Fisher's Exact Test: $p = 0.46$). At D14, all testosterone-treated males but none of the six control-treated males were scrotal (Fisher's Exact Test: $p = 0.002$).

Testosterone treatment was significantly associated with testis mass (GLM: $F_{1,10} = 19.58$; $p = 0.001$) and with epididymis mass (GLM: $F_{1,10} = 116.36$; $p < 0.001$), both being heavier in testosterone-treated males (Table 1).

Testes of testosterone-treated males had, though not quite significantly, greater haploid signals (1C: $W = 32.5$; $p = 0.06$), significantly lower diploid signals (2C: $W = 2$; $p = 0.009$) significantly greater tetraploid signals (4C: $W = 34$; $p = 0.009$), and more cells in the S-phase (S: $W = 31$; $p = 0.04$) than testis of control-treated males (Table 2). The haploid/diploid ratio (1C/2C) was higher, though not significantly, for the testis of testosterone-treated males than those of control-treated males ($W = 29$; $p = 0.08$). No significant difference was observed for the haploid/tetraploid ratio (1C/4C) ($W = 24$; $p = 0.34$). We found a significantly lower diploid/tetraploid ratio (2C/4C) in the testis of testosterone-treated males than of control-treated males ($W = 2$; $p = 0.009$). Spermatozoa were only found in the testis of a single testosterone-treated male. This male was the only male with a 1C/4C ratio greater than one (1.6).

3.7. Home ranges and sleeping sites

Testosterone-treated males had on average twice as large home ranges as control-treated males (0.60 ± 0.25 ha vs. 0.28 ± 0.08 ha; $W = 31$; $p = 0.045$) (Fig. 3). Home range sizes of testosterone-treated males did not differ significantly from those of dominant group-living territorial breeders (0.60 ± 0.25 ha vs. 0.75 ± 0.87 ha; $W = 25$; $p = 0.62$). Although they were about one third the size, home range sizes of control-treated males did not differ significantly from those of dominant group-living territorial breeders (0.28 ± 0.08 ha vs. 0.75 ± 0.87 ha; $W = 11.5$; $p = 0.20$).

Both testosterone-treated and control-treated males slept most of the time with another member of the group between D-1 and D14 (93.55 ± 2.17% vs. 98.39 ± 1.02%; GLM: $F_{1,11} = 2.87$; $p = 0.12$).

4. Discussion

To date, few studies have demonstrated that an experimental increase of circulating testosterone levels can influence which reproductive tactic an individual will follow or that it can accelerate the development of a specific reproductive tactic [23]. According to the high testosterone levels observed in solitary-living roamers [36,38], we predicted that juvenile males with an experimentally-induced increase in serum testosterone levels during the breeding season would undergo morphological, physiological, and behavioral changes indicating the onset of puberty. We observed that testosterone implants led to changes in corticosterone levels, accelerated testes development, activated spermatogenesis, and increased home range sizes. Testosterone-treated males had no opportunity to become the dominant group-living territorial breeder of another group, as their body mass was only 50% of the dominant breeders. Importantly, body mass determines competitive ability and thus reproductive tactics in African striped mice [34,35,38]. However, they could have left their natal group and become solitary living roamers, which they did not, indicating that the onset of the roaming tactic was incomplete.

Male puberty occurs when gonadotropin-releasing hormone and gonadotropin secretion increases [3] resulting in gonadal testosterone surges, which leads to sexual maturation [22]. Juvenile males with an experimental increase in serum testosterone levels became scrotal and had heavier testes and epididymides than the non-scrotal control-treated males. This demonstrates that our testosterone treatment induced sexual maturation in juvenile males.

Table 1
Body mass of the testosterone- and control-treated males at D-1, D6, and D14, and testis and epididymis mass presented as the percentage of the body mass of the testosterone- and control-treated males. Mean ± SD.

	Body mass (g)			Testis (%)	Epididymis (%)
	D-1	D6	D14		
Test males	22.0 ± 3.6 ($N = 7$)	23.7 ± 3.4 ($N = 7$)	25.8 ± 3.1 ($N = 5$)	0.26 ± 0.04 ($N = 6$)	0.044 ± 0.005 ($N = 6$)
Control males	22.3 ± 4.1 ($N = 6$)	23.0 ± 4.9 ($N = 6$)	25.0 ± 4.6 ($N = 6$)	0.08 ± 0.02 ($N = 6$)	0.012 ± 0.002 ($N = 6$)

Table 2
Ploidy-state of the testis tissues of testosterone- and control-treated males. %1C, %2C, %4C, and %S are the percentages of haploid, diploid, and tetraploid signals as well as cells in the S-phase. 2C/4C, 1C/4C, and 1C/2C are the signal ratios. Mean ± SD.

Signals and ratios	Test males ($N = 6$)	Control males ($N = 6$)
%1C	9.56 ± 12.38	0.57 ± 0.36
%2C	56.23 ± 26.01	84.98 ± 4.50
%S	9.73 ± .90	6.29 ± 1.18
%4C	22.78 ± 12.40	6.87 ± 3.44
2C/4C	4.12 ± 4.52	14.85 ± 6.22
1C/4C	0.40 ± 0.60	0.09 ± 0.06
1C/2C	0.39 ± 0.60	0.01 ± 0.00

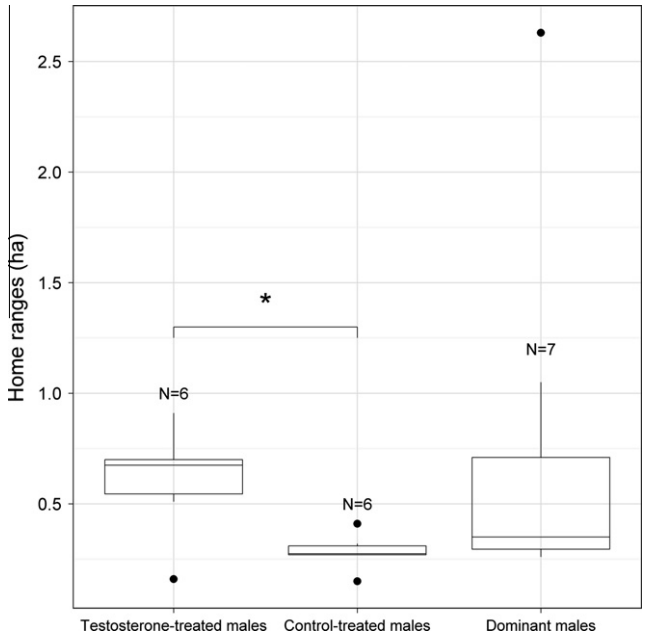

Fig. 3. Home range sizes (mean ± SD) after 8 days of radiotracking for testosterone-treated males (grey), control-treated males (white), and dominant group-living territorial breeders (black). The median is indicated by the horizontal bar inside the box, the first and third quartile by the box itself, and the horizontal bars outside the box indicate the minimal values and the fourth quartile (outliers are shown by a black-filled circle). Only significant pairwise comparisons are reported on the figure: $^{*}p < 0.05$.

Furthermore, we found evidence that an increase in circulating testosterone levels activated spermatogenesis in juvenile males. This is in agreement with the general pattern in male vertebrates, where increased circulating testosterone levels are known to enhance the development of external (i.e. scrotal development) and internal reproductive structures (i.e. testicular development), as well as spermatogenesis [22].

Spermatogenesis can be studied by measuring the DNA content of cells in the testes [5]. Haploid signals (1C) originating from round spermatids or spermatozoa were observed more often in the testes of testosterone-treated males than those of control-treated males, which showed almost no haploid signals. But spermatozoa were microscopically observed in only one test male. This juvenile male was the only one with a ratio of haploid to tetraploid signals greater than one, which is a measure of the meiotic yield from primary spermatocytes to the haploid product. Since the duration of spermatogenesis in laboratory mice (*Mus musculus domesticus*) is about 35 days [7], an experimental increase of plasma testosterone levels of 15 days might have been too short to lead to complete spermatogenesis in testosterone-treated males. Importantly, the meiotic and/or mitotic activity was greater in the testes of testosterone-treated males than of control-treated males (inferred from lower diploid/tetraploid ratio in testosterone-treated males). A high proportion of haploid nuclei indicate meiotic activity and the onset of sperm production, at least to the stage of round spermatids. This was indicated by a higher haploid/diploid ratio in test males. Both results are indicative of the occurrence of meiosis in testosterone-treated males but not in control-treated males. We conclude that spermatogenetic activity began in the testes of testosterone-treated males but not of control-treated males.

In African striped mice, juvenile male kept individually in captive condition are typically sexually mature (scrotal) when being 3–4 weeks old [33,39]. In contrast, juvenile males kept in family group are sexually mature when being 4–5 weeks old (the age of juvenile males used in the present study at the start of our testosterone treatment) [33,39]. In fact, juvenile males remaining in their family group delay sexual maturation [42] due to environmentally- and socially-induced sexual suppression [39]. High corticosterone levels might be the endocrine mechanism of this suppression [38]. Interestingly, testosterone-treated males showed lower basal corticosterone levels than control-treated males after (but not before) the treatment. Central and peripheral actions of testosterone leading to decreased glucocorticoid levels have been described in several previous studies [17,18,30,49]. Thus, the testosterone treatment had likely suppressive effects on the activity of the HPA axis. The resulting decrease of corticosterone secretion from the adrenal gland might have lifted the inhibition of gonadal functions, especially spermatogenesis, in testosterone-treated males, but not in control-treated males. Our results indicate that an interaction between testosterone and corticosterone might regulate the reproductive capacity of juvenile males [29]. Taken together, the faster gonad development and the activation of gonadal function induced by the increase of testosterone levels demonstrate that testosterone-treated males underwent sexual maturation, which was inhibited or at least delayed in control-treated males.

In the present study, the silastic testosterone implants increased testosterone levels to the upper physiological range of testosterone observed in solitary-living roamers (9 ng/ml in Schradin and Yuen [36]). Experimental increase of testosterone levels led to significant changes in the physiological and reproductive status of juvenile males. However, testosterone-treated males remained as philopatric group-living males, just as control males. Why did testosterone-treated males not leave their natal group to become solitary-living roamers? We observed that testosterone-treated males had larger home ranges than control males. Testosterone can reduce anxiety in laboratory male mice [1] and it is known from other species that experimental testosterone administration can lead to larger home range sizes [6,10,19,50]. Increasing home range size might help individuals to gather information about when and where to disperse. However, the decision to disperse and to become solitary-living roamers or to stay in the natal group as philopatric group-living males may also depend on environmental conditions. During our experiment, five out of 19 adult females were breeding solitarily and most adult males were dominant group-living territorial breeders. Schradin and Lindholm [35] predicted that philopatric group living males would make the decision to remain group-living philopatric males and not to disperse when the ratio of solitary- to group-living females is below 1, as it was in our study. Furthermore, under normal environmental conditions 100% of females reproduce during the breeding season [35], but extremely low rainfall during our study resulting in low food availability might have been the reason why only 42% of females reproduced. This limited the chances for solitary-living roamers to reproduce, and might explain why only one solitary-living roamer was present in the population. The specific environmental conditions of our field study, i.e. the few mating opportunities, could explain why testosterone-treated juvenile males did not

disperse and did not become solitary-living roamers. In sum, increased testosterone and decreased corticosterone levels were sufficient to induce sexual maturation, but were not sufficient to elicit the development into the solitary-living tactic.

5. Conclusion

To our knowledge, this is the first study experimentally testing the activational effects of testosterone on the induction of sexual maturation and the onset of ARTs in a free-ranging mammal species. We observed that the experimental increase of testosterone levels accelerated sexual maturation but did not lead to dispersal and solitary-living. The larger home ranges of testosterone-treated males could be due to testosterone-induced anxiolytic effects [1], allowing testosterone-treated males to assess the prevailing environmental conditions. However, the environmental conditions of our study were not favorable for dispersal, as reproductive opportunities were few [35]. This could explain why juvenile males hormonally primed to reproduce did not become solitary-living roamers. Therefore, we recommend that future studies consider the role of environmental cues in addition to hormonal signals in the neuroendocrine control of ARTs.

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